

Changes in Epstein-Barr Virus Antibody Titers Associated with Aging (42108)

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Abstract. Antibody titers to the Epstein-Barr virus (EBV), early antigen (EA) IgG, and virus capsid antigen (VCA) IgG and IgA, were measured in 44 geriatric subjects to determine if the depression in cellular immunity known to be associated with aging affects the expression of latent EBV. Similar assays were performed on plasma obtained from a young adult (medical student) population as a control group. We found that 89% of the geriatric samples were positive for EA IgG, and 83% of the plasma obtained from medical students were positive for EA IgG. One hundred percent of the geriatric subjects were positive for VCA IgG, and 87% of the medical students were positive for VCA IgG. Seven percent of the medical student blood samples were positive for VCA IgA; in contrast, 36% of the blood samples obtained from the geriatrics subjects were positive. Significant differences were also found in the geometric mean titers (GMT) of antibodies to EBV antigens; the GMT to EBV EA and VCA were significantly higher in the geriatric group. The data suggest that there may be some loss of control over latent EBV by the cellular immune response in geriatric individuals. © 1985 Society for Experimental Biology and Medicine.

Studies on humans and laboratory animals have suggested that immune functions generally decline with age. It is known that T-lymphocyte-mediated immune function decreases while the production of certain autoantibodies, (e.g., antinuclear antibodies) tends to increase with aging (1-3). There are also data suggesting that, while there is no change in levels of immunoglobulin M (IgM) in plasma, there is an increase in immunoglobulin G (IgG) and immunoglobulin A (IgA) in older individuals (4).

In this study, we examined levels of IgG and IgA antibodies to a latent herpesvirus in a geriatric population to determine if there were any differences in the antibody patterns in these individuals when compared to a younger population. We assayed for antibody titers against the Epstein-Barr Virus (EBV) early antigen (EA) and virus capsid antigen (VCA). The EBV is a human herpesvirus known to be the etiological agent for infectious mononucleosis (IM) (5); it has also been closely associated with nasopharyngeal carcinoma (NPC) and Burkitt's lymphoma

(BL) (6). It is of interest that VCA antibody of the IgA class is present in a high percentage of NPC patients, and antibody titers to VCA, IgG, and IgA often increase with the stage of disease in such patients (7-10).

The specificity of IgA antibodies to NPC has been explored in some detail, as already discussed, but the presence of IgA antibodies in normal individuals has not been extensively explored. In this study, we determined the antibody titers to EBV VCA, IgG, and IgA and EA IgG in normal geriatric subjects who were residents of independent living facilities. We found that there were significantly higher antibody titers to all EBV antigens including anti-VCA IgA in this geriatric population.

Subjects and methods. *Subjects.* Forty-four geriatric patients from three independent living facilities in Ohio were examined for antibody to EBV EA and VCA IgG and VCA IgA. To make age-related comparisons, antibody titers from plasma samples taken from 70 medical students in their second year at the Ohio State University College of Medicine were also determined. The mean age of the geriatric sample was 72, with a range from 62 to 90, and the mean age of

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the students was 23, with a range from 22 to 27.

Cell lines. The P3J HR-1 (HR-1) producer BL cell line and the nonproducer Raji BL cell line were grown in RPMI 1640 medium supplemented with 8% fetal bovine serum. The cells were maintained at 35°C.

Immunofluorescence assays. IgG antibody titers to EA were determined using acetone-fixed cell smears of Raji cells superinfected with concentrated filtered EBV derived from the HR-1 cell line by the procedure already described (11). The HR-1 superinfected Raji cells were incubated at 35°C in the presence of 5 µg/ml cytosine arabinoside (Ara-C) for 24 hr (to inhibit VCA synthesis) and then fixed for determination of EA antibody titers. Smears of HR-1 cells were used to detect VCA IgG and IgA antibody titers. Smears of HR-1 cells were used to determine VCA IgG and IgA antibody titers. Cells were adsorbed with twofold dilutions of plasma in phosphate-buffered saline (PBS), pH 7.4, for 30 min at 37°C in a plastic chamber, washed twice with PBS, and readsorbed with anti-human IgG (goat) conjugated to fluorescein isothiocyanate (FITC) (Cappel Laboratories). The cells were incubated for an additional 30 min at 37°C, washed three times with PBS, counterstained with 0.05% Evans blue, mounted in Protex, and examined by a Zeiss ultraviolet light microscope. All slides were read blind coded. Assays for VCA IgA antibody titers were the same as for VCA IgG except that the second adsorption involved the use of goat anti-human IgA FITC (Cappel Laboratories). Plasma was used rather than serum since it was necessary to obtain heparinized blood for other studies. No differences in antibody titers were observed in experiments using plasma and serum from the same individuals.

Results. The percentage of individuals positive for each antibody, and the geometric mean titers (GMT) for each antibody in each group, were determined. As shown in Table I, 89% of the geriatric samples were positive for EA IgG, and 83% of the medical students were positive for EA IgG. One hundred percent of the geriatric samples were positive for VCA IgG, and 87% of the medical students had antibody to VCA IgG. We found

TABLE I. PERCENTAGE INDIVIDUALS POSITIVE FOR ANTIBODY TO EBV ANTIGENS

Population and total No. samples assayed	EBV antibody		
	EA (IgG)	VCA (IgG)	VCA (IgA)
Geriatric (44)	89	100	36
Students; young adults (70)	83	87	7

that approximately 7% of the plasma samples obtained from the medical students were positive for VCA IgA antibody. However, 36% of the geriatric samples were found to be positive.

Since the method of doubling dilutions had been used to obtain the EBV antibody titers, a base 2 logarithmic conversion was used to reduce variance for the statistical comparisons (12). However, for descriptive purposes GMTs are presented to allow comparisons with previous research. As shown in Table II, the students' EA GMT of 53 was significantly lower than the EA GMT of 459 in the geriatric group, $F(1, 104) = 68.94$, $P < 0.0001$, as determined by using a one-way analysis of variance. Similarly, there was a significant difference between the VCA IgG GMT of 94 for the student sample, compared to 662 for the geriatric sample, $F(1, 96) = 12.38$, $P < 0.0007$, in subjects who were VCA positive.

The very small number of students seropositive for IgA made GMT comparisons between the two groups of little value; however, a χ^2 analysis was used to compare the relative frequency of IgA positive subjects who were EBV seropositive in the two groups. There was a significant difference in the relative frequency of IgA positive subjects, $\chi^2 (1, N = 105)$, $P < 0.0001$, with significantly more positive subjects in the geriatric population.

Discussion. At no time during the course of the study did anyone complain of symptoms compatible with IM, or persistent EBV infections, as recently described (13). The higher levels of antibody in the geriatric group were not associated with clinical disease. Presumably, the participants who were

TABLE II. GMT OF ANTIBODY TO EBV ANTIGENS
IN A YOUNG ADULT POPULATION AND
A GERIATRIC POPULATION

Population	EBV antibody		
	EA (IgG)	VCA (IgG)	VCA (IgA)
Geriatric	459	662	17
Students (young adults)	53	94	3.6

EBV positive had been previously infected with EBV. Characteristic of this type of individual is the absence of VCA specific IgM antibodies, usually moderate titers of VCA IgG antibodies, low levels of EA antibodies, as well as the presence of antibody to the EBV nuclear antibody (EBNA) (14).

In a previous study of NPC patients for VCA IgA antibodies by Ho *et al.* (15) no VCA IgA antibodies were found in any of the control subjects. In another study, less than 5% of sera from control individuals were positive for IgA antibody to EBV VCA (8). Similar data were obtained in a recent study of Chinese NPC patients when the controls were examined (10).

The statistical analyses of the GMTs between the geriatric and student groups in this study show clear significant differences in regard to EA and VCA IgG GMTs, as well as the relative frequency of VCA IgA positive individuals. Though the geriatric population studied is relatively small (44), the data suggest that by the age of 72 (the average age of this group) all individuals had been infected with EBV. The data also suggest that there may be some loss of control over latent EBV in geriatric individuals, presumably by the cellular immune response. This could be related to the depression in T-lymphocyte-mediated immune functions already discussed (1-3). It is of interest that changes in EBV specific antibody titers were recently found to be affected by stress (16). It was suggested that these changes were also associated with a depression in cellular immunity. Thus, studies concerned with linking EBV with malignancy which use EBV antibody titers as a measure should take into account the age of the individuals being assayed.

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